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A preliminary investigation into the use of biosensors to screen stomach contents for selected poisons and drugs

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Abstract

The bioluminescence response of two genetically modified (*lux*-marked) bacteria to potentially toxic compounds (PTCs) in stomach contents was monitored using an *in vitro* assay. Cells of *Escherichia coli* HB101 and *Salmonella typhimurium* both carrying the *lux* light producing gene on a plasmid (pUDC607) were added to stomach contents containing various concentrations of organic and inorganic compounds. There was some variability in the response of the two biosensors, but both were sensitive to the herbicides glyphosate, 2,4-dichlorophenoxyacetic acid (2,4-D), 2,4,5-trichlorophenoxyacetic acid (2,4,5-T); pentachlorophenol (PCP), and inorganic poisons arsenic and mercury at a concentration range likely to be found in stomach contents samples submitted for toxicological analysis. This study demonstrates that biosensor bioassays could be a useful preliminary screening tool in forensic toxicology and that such a toxicological screening should include more than one test organism to maximise the number of PTC's detected. The probability of false positive results from samples containing compounds that may interfere with the assay such as over-the-counter (OTC) drugs and caffeine in tea and coffee was also investigated. Of the substances tested only coffee has the potential to cause false positive results.
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1. Introduction

Forensic toxicologists are frequently asked to implicate or exclude chemical poisoning in a suspicious death or unexplained illness. The range of analyses performed generally depends on the circumstances of the case. Primary tests usually include screens for a wide range of medicinal and illicit drugs and possibly tests for carbon monoxide, cyanide and toxic metals. Subsequent analyses which focus on individual poisons or groups of poisons are generally very time-consuming. Any procedure which could screen for a range of such PTC's could therefore save significant analytical time.

A bacterial bioassay was developed to screen for a variety of potentially toxic chemicals in urine [1]. An *Escherichia coli* bacterium has been genetically modified with light producing genes (the *lux* gene cassette) that, under normal conditions give out visible light (bioluminesce) [2,3]. The light output is directly proportional to the metabolic activity of the biosensor and any chemical that causes metabolic stress to the bacterial cell will result in a decline in luminescence that is directly related to the concentration of toxic compound present. This gives a measure of the overall toxicity of a sample and requires no pre-knowledge about the nature of toxicity in the sample (as is necessary when employing standard chemical analysis). Preliminary investigations have shown that this biosensor can indicate the presence of a variety of herbicides such as glyphosate, pentachlorophenol (PCP), 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T); and inorganic poisons such as arsenic, mercury and cyanide in urine [1].

Toxicological samples received by forensic laboratories often include stomach contents. A system for screening such samples would be useful to assist in the determination of cause of death in instances where a urine sample is unavailable, or levels of the drug or poison in urine are too low to be detected by the *E. coli* biosensor (e.g. in rapid deaths).

In this paper we describe further development of an *E. coli* HB101 pUCD607 biosensor to screen stomach contents for the

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presence of drugs and poisons. The *E. coli* biosensor may not be ecologically appropriate for stomach contents testing (i.e. the stomach contents environment may not be optimum for the *E. coli* biosensor) so we also describe construction of a *lux* modified *Salmonella typhimurium* (STM) for use as a ''specific'' biosensor for stomach contents screening. *Salmonella* sp. are one of the leading causes of food borne disease [4,5], and consequently can survive passage through the stomach. Thus, *Salmonellae* may be ideal organisms for use as biosensors in stomach contents. Methanol extraction of organic PTC's was trialled as a possible method to increase biosensor sensitivity and also as a way to determine if an unknown PTC is organic or inorganic.

The effects of common over-the-counter (OTC) compounds such as ibuprofen, and of compounds such as caffeine were also investigated. These compounds may also be toxic to the biosensor, potentially creating false positive results. For this reason, commonly encountered therapeutic compounds were screened to determine their toxicity towards the *E. coli* or STM biosensors.

2. Materials and methods

2.1. Bacterial growth conditions

The STM used in this study was a clinical isolate provided by the Enteric Reference Laboratory at ESR (New Zealand). It was routinely grown in brain heart infusion (BHI) broth (BHIB; Difco) at 37 °C with shaking at 200 rev/min [6]. Antibiotic resistance characterization showed that this isolate was tetracycline-resistant and ampicillin, spectomycin and kanamycin-susceptible. *E. coli* HB101 pUCD607 was routinely cultured in Luria–Bertani (LB) broth (10 g tryptone, 5 g yeast extract, 10 g NaCl, 1 L distilled water) at 25 °C shaking at 200 rev/min.

2.2. Construction of lux-marked STM

The *lux*-marking of STM bacterium involves transfer of the plasmid carrying the *lux* genes from the donor *E. coli* HB101 pUCD607. Plasmid pUCD607 contains the *Vibrio fischeri lux*CDABE genes and three antibiotic resistance genes (for ampicillin, spectomycin and kanamycin resistance). The pUCD607 plasmid was purified using an ABI Prism Miniprep Kit (Applied BioSystems).

The STM bacterium must be made 'competent' to take up extra plasmid DNA [7]. Competent cells were prepared by growing a STM culture in BHI broth to a density of 10^8 cells/mL. Fifty milliliters of cells were then transferred to a 50 mL sterile, ice-cold centrifuge tube to cool the culture. The cells were recovered by centrifugation at 3000 × *g* for 10 min at 4 °C. The pellet was then re-suspended in 10 mL of ice cold 0.1 M $CaCl_2$ and stored on ice. The cells were once again centrifuged at 3000 × *g* for 10 min at 4 °C and re-suspended in 2 mL of ice cold 0.1 M $CaCl_2$ for each 50 mL of original culture.

Competent STM cells (200 μL) were transferred to a microcentrifuge tube and 50 μL of plasmid DNA was added and mixed by gentle swirling. The tubes were then stored on ice for 30 min. The tubes were then placed in a water bath at 42 °C for 90 s, and then rapidly transferred to an ice bath and allowed to chill for 1–2 min. Eight hundred microliters of SOC medium (20 g tryptone, 5 g yeast extract, 0.5 g NaCl, 2.5 mL 1 M KCl, 1 L distilled water) was added to each tube and the tubes were incubated for a further 45 min in a water bath at 37 °C. Two hundred microliters aliquots were then plated out onto LB agar plates (plus tetracycline and kanamycin) and incubated overnight. The following day the plates were examined for transformants. The STM transformants containing the pUCD607 plasmid were confirmed by detecting luminescence. Brightly luminescing colonies were purified by streaking onto LB agar plates (plus tetracycline and kanamycin). The transformed strain was designated *S. typhimurium* ER0000483STI pUCD607 (STM *lux*) and aliquots stored in 10% glycerol at −80 °C.

2.3. Preparation of stomach contents

The stomach contents were provided by ESR Toxicology Laboratory. Stomach contents from 50 deceased individuals were pre-screened according to our standard operating procedures (ESR Toxicology Laboratory) and found to contain no drugs or toxins. These samples were stored frozen until required. Individual stomach contents samples were defrosted and centrifuged at 3000 × *g* for 1 h to remove particulate matter, then adjusted to pH 7 using 1 M NaOH. pH adjustment was required in order for the biosensor to survive and remain active in the sample.

2.4. Preparation of PTC standards in stomach contents

The PTCs selected for testing represented compounds to which the *E. coli* biosensor had previously demonstrated sensitivity [1], are encountered relatively frequently by toxicologists, and/or can only be detected by individually tailored and time consuming tests. The PTCs were: the herbicide ''Roundup®'' containing *N*-(phosphonomethyl) glycine (glyphosate), 2,4-D (sodium salt), 2,4,5-T (sodium salt), sodium cyanide, sodium arsenate, sodium arsenite, mercuric chloride and PCP. Stock solutions were made up as follows: the appropriate amount of each of the chemicals was individually weighed out using a 5-point balance and transferred to acid washed 5 mL glass volumetric flasks and made up to the mark with stomach contents. Test solutions were prepared by diluting the stock solutions to the required concentration with stomach contents.

With the exception of 2,4-D, 2,4,5-T and PCP, the PTC's dissolved readily. These compounds were pre-dissolved in water as follows: (1) 2,4-D. Add 10 M NaOH whilst stirring until dissolved (for 10,000 ppm in 25 mL five drops 10 M NaOH were required). (2) 2,4,5-T. As with 2,4-D, only four drops of 10 M NaOH required. (3) PCP. Pre-dissolve in 100% methanol to a final concentration of 2% methanol. These solutions were then diluted, to the required concentrations, with stomach contents.

2.5. Methanol extraction

For each of the organic PTCs tested (Round-up, 2,4-D, 2,5,5-T and PCP), methanol extraction was also carried out. Stock solutions of each concentration of PTC were diluted with stomach contents. Each of these standards (4 mL) was made up to 20 mL with methanol and mixed well. The samples were then centrifuged at 3000 × *g* for 5 min and then the supernatant was placed in a hot block and dried under a gentle stream of nitrogen gas (at 40 °C). Dried samples were re-suspended in 4 mL of double distilled water and used directly in the bioassay.

2.6. Preparation of OTC drug standards

A range of commonly encountered OTC drugs were selected for testing: codeine phosphate, pseudoephedrine hypochloride, chloramphenicol, promethazine, cyclizine HCl, pholcodine, paracetamol and acetylsalicylic acid (Aspirin). Stock solutions in double distilled water were prepared for each OTC drug at a concentration approximately 10 times that normally expected to be found in stomach fluids when taken in therapeutic doses as prescribed. Note that 'normal' levels of OTC drugs were estimates of the concentration expected in stomach contents if the subject had taken the medication according to the manufacturer's recommended dose shortly before death [8–10].

Most compounds dissolved at this concentration readily (at room temperature), exceptions being: (1) Aspirin; where solubility was achieved by increasing the temperature to 50 °C, stirring continuously for 1 h. (2) Paracetamol; which was pre-dissolved in 2% ethanol. (3) Pholcodine; which was pre-dissolved in 2% ethanol. Bioassays were carried out initially in water (Table 2), then in stomach contents if the results indicated toxicity of the OTC drug towards the biosensor.

2.7. Preparation of caffeine

The toxicity of caffeine and caffeine containing beverages to the biosensors was also investigated. A stock solution of caffeine was made up as for the other PTC's and test solutions were prepared by diluting the stock solution to the required concentration with water and stomach contents. Filter coffee and tea were prepared and the caffeine concentrations determined by gas chromatography–mass spectrometry. The concentration of caffeine in the filter coffee used was 540 mg/L, for the tea it was 370 mg/L. A standard cup of coffee contains 50–150 mg of caffeine [10]. The prepared coffee and tea were diluted to the required concentrations with water and stomach contents.

2.8. Preparation of the STM biosensor

For each assay, a culture of STM was grown in 10 mL of LB broth with kanamycin (1 mg/mL) in a 20 mL thin walled Universal bottle shaken at 180 rev/min at 25 °C until a cell concentration of 10^8 cfu/mL was obtained (optical density of 0.8). The cells were centrifuged at 7550 × *g* for 1 min. The supernatant fluid was discarded and the pellet resuspended in half the original volume of Ringers solution (Difco). Immediately before use, the bioluminescence of 100 μL of the resuspended cells was measured in a luminometer (LumiSkan TL, Labsystems) to evaluate the effect of stomach contents on luminescence.

A suspension of cells (100 μL) was added to 900 μL of test solution and mixed by pipetting up and down for 5 s, at 10 s intervals between each sample. Luminescence was measured in a LumiSkan TL (Labsystems) after a 15 min exposure. All assays were carried out in triplicate using independent vials. Data were expressed as a percentage of the luminescence measured in control samples.

2.9. Quality assurance of biosensors

To assure the quality of the response, the *E*. coli and STM biosensors were exposed to test solutions containing serial dilutions of TCP™ liquid antiseptic (Pfizer Ltd., UK), an aqueous glycerol solution of halogenated phenols (0.68%, w/v) and phenol (0.175%, w/v). The effect of stomach contents on the biosensor's response was determined by comparing the TCP™ EC_{50} in both stomach contents and water. For both biosensors this was carried out in triplicate with three different samples of stomach contents to observe any variation in biosensor response.

2.10. Data interpretation

Bioluminescence was plotted as a percentage of the response of the biosensor to the unadulterated stomach contents (control) against PTC concentration. Negative exponential curves were fitted to the data points by non-linear regression techniques for most of the PTCs. The decline in bioluminescence was described by the negative exponential curve, $Y = a + be^{-kx}$, where Y = % bioluminescence, X = PTC concentration, a = lower asymptote, b = range of Y values, K = rate parameter [11]. Curves were fitted using Prism 3.0 (San Diego, USA) and EC_{50} values were calculated by solving the equation for values of $Y = 50\%$ for each of the PTCs.

Table 1
EC_{50} values for *E. coli* and STM biosensors exposed to TCP™ in three different stomach contents

Stomach contents	*E. coli* biosensor response (EC_{50})	STM biosensor response (EC_{50})
1	0.53	1.2
2	0.65	1.5
3	0.77	2.2
Average (±S.D.s)	0.65 (±0.12)	1.63 (±0.51)

3. Results

3.1. Quality assurance of biosensors

Cells of both *E. coli* and STM remained stable for 1 h after preparation (data not shown). Exposure of the bacterial biosensors to stomach contents at pH's below 7 markedly reduced bioluminescence (data not shown). However, pH adjustment of the stomach contents to 7 did not significantly decrease bioluminescence, indeed in some instances bioluminescence was increased on exposure to ''control'' (unspiked with PTC) stomach contents.

The negative exponential curves fitted to the data points by non-linear regression techniques had R^2 values of greater than 0.8 with $P < 0.05$ in all cases. TCP™ concentrations that caused a 50% decline in bioluminescence (EC_{50} value) for the *E. coli* and STM biosensors in water were 0.28 and 0.45 ppm, respectively. EC_{50} for the TCP™ test solutions in stomach contents (triplicate results) are shown in Table 1. Although stomach contents can be highly variable, the EC_{50} values were very similar and an analysis of variance of the three data sets showed no significant difference ($P = 0.82$ for the *E. coli* biosensor and 0.43 for STM biosensor results). For both biosensors, the EC_{50} values for the TCP™ test solutions were higher in stomach contents than in water.

Table 2
Response of the *E. coli* and STM Biosensors to a range of potentially toxic compounds (PTC)

PTC	Typical stomach contents (minimum or average) concentrations (mg/L) in fatal poisonings	References	*E. coli* biosensor response (EC_{50})	*E. coli* biosensor response (EC_{50}) to methanol extracted PTC	Detectable using *E. coli* biosensor (✓/×)	STM biosensor response (EC_{50})	STM biosensor response (EC_{50}) to methanol extracted PTC	Detectable using STM biosensor (✓/×)
Round-up	35000 (minimum)	[12]	2457	913	✓	6110	686	✓
2,4-D	70000 (minimum)	[9]	612	658	✓	714	580	✓
2,4,5-T	>70000 (minimum[a])	[9]	286	548	✓	716	568	✓
PCP	2000 (average)	[8]	11	8.6	✓	68	35	✓
Cyanide	100 (minimum)	[8]	30	–	✓	150	–	×
Arsenate	>70 (minimum[b])	[9]	>500	–	×	165	–	×
Arsenite	70 (minimum)	[9]	89	–	✓/×	4.6	–	✓
Mercuric	1000 (average)	[8]	64	–	✓	73	–	✓

[a] Toxicity is assumed to be similar to 2,4-D.
[b] Pentavalent (arsenate) is less toxic than trivalent (arsenite) [9].

3.2. Response of biosensors to PTC standards in stomach contents

The bioluminescence response of the *lux*-modified *E. coli* and STM biosensors to a range of PTCs in stomach contents was characterised and EC_{50} values were calculated (Table 2). Results showed that the biosensors provide appropriate sensitivity for testing of a broad spectrum of PTCs in stomach contents. The biosensors were sensitive to Round-up, 2,4,5-T, 2,4-D, PCP, arsenite and mercury at concentrations likely to be found in stomach contents in cases of fatal poisonings. Toxicity of cyanide towards the two biosensors varied. For the STM biosensor, the EC_{50} value was higher than the typical concentration of cyanide in stomach contents in a fatal poisoning (100 ppm). The *E. coli* biosensor was more sensitive to cyanide with an EC_{50} value of 30 ppm (Table 2).

3.3. Methanol extraction

Methanol extraction of the organic PTC's from spiked stomach contents increased the sensitivity of the biosensor response for Round-up (Table 2). The EC_{50} value decreased from 2457 ppm in stomach contents, to 912 ppm in methanol extracted samples for the *E. coli* biosensor; and decreased from 6110 to 686 ppm for the STM biosensor. The EC_{50} values for all the other organic compounds tested were higher for the methanol extracted samples, than for direct exposure to the PTC in stomach contents.

3.4. Response of biosensors to OTC drug standards

Dose response curves were plotted of bioluminescence against the OTC drug concentrations in water for both the *E. coli* and STM biosensors. EC_{50} values were calculated from the dose response curves and are shown in Table 3. Results in Table 3 indicate that the majority of OTC drugs tested were not toxic to the biosensors at therapeutic doses. Exceptions were promethazine which had EC_{50} values lower than the therapeutic dose on exposure to the *E. coli* biosensor; and Aspirin that was toxic to both biosensors. However, when bioassays were repeated in stomach contents, sensitivity of the biosensors to the OTC drugs was reduced to levels above the therapeutic dose (Table 3).

3.5. Caffeine

Dose response curves were plotted of bioluminescence against coffee, tea and caffeine in water and stomach contents for both the *E. coli* and STM biosensors. EC_{50} values were calculated from the dose response curves and are shown in Table 3. The coffee and tea prepared for this study were toxic to both biosensors. The EC_{50} values for both biosensors were reached with dilutions of both beverages (Table 3). For the *E. coli* biosensor, the concentration of caffeine in coffee and tea that caused a 50% reduction in bioluminescence was 33 and 27 ppm, respectively. The STM biosensor was less sensitive with EC_{50} values for caffeine in coffee and tea of 89 and

Table 3
Response of the *E. coli* and STM biosensors to over-the-counter (OTC) drugs and caffeine

OTC drug	Normal levels[a] (mg/L)	References	*E. coli* biosensor response (EC_{50}) in water	Detectable using *E. coli* biosensor (✓/×)	*E. coli* biosensor response (EC_{50}) in stomach contents	Interference with bioassay? (✓/×)	STM biosensor response (EC_{50}) in water	Detectable using STM biosensor (✓/×)	STM biosensor response (EC_{50}) in stomach contents	Interference with bioassay? (✓/×)
Promethazine	50	[8]	23	✓	574	×	55	✓	313	×
Pseudoephedrine	60	[8]	>6000	×	N/A	×	>6000	×	N/A	×
Chlorpheniramine	12	[9]	>1000	×	N/A	×	>1000	×	N/A	×
Pholcodine	10	[8]	36	×/✓	>1000	×	>2000	×	>2000	×
Cyclizine	50	[9]	139	×	N/A	×	193	×	N/A	×
Aspirin	325	[8]	78	✓	1658	×	7.3	✓	2020	×
Codeine	60	[8]	>1000	×	N/A	×	>1000	×	N/A	×
Paracetamol	1000	[9]	3759	×	N/A	×	7299	×	N/A	×
Coffee	50–150 (as caffeine)	[10]	33 (14% coffee[b])	✓	133 (25% coffee[b])	✓	89 (as caffeine)	✓	86 (as caffeine)	✓
Caffeine	32–200	[10]	294	✓	>3000	×	251	✓	2503	×
Tea	40–150 (as caffeine)	[10]	27 (13% tea[b])	✓	188 (50% tea[b])	×	160 (80% tea[b])	×	N/A	×

+EC_{50} is close to potential levels in stomach contents.
[a] "Normal" levels were estimates of the concentration expected in stomach contents if the subject had taken the medication according to the manufacturer's recommended dose shortly before death.
[b] The toxicity of caffeine to the biosensors is expressed as ppm caffeine and the corresponding dilution (as % of neat coffee or tea) tested.

161 ppm, respectively. Both biosensors were less sensitive to pure caffeine (Table 3). When the bioassays were repeated in stomach contents, only coffee gave EC_{50} values below the levels of caffeine potentially in stomach contents if a subject had just drunk a cup of coffee before death.

4. Discussion

The biosensors were able to indicate the presence of a select number of toxic substances in stomach contents at concentrations likely to be found in toxicological samples. However, exposure of the biosensors to stomach contents that had not been pH adjusted caused a rapid decline in luminescence. Previous studies using the biosensors in environmental samples such as soil solution have shown the luminescence response from the biosensors to be stable across the pH range 4.5–7.0 [13]. It is possible that the presence of hydrolytic enzymes in the stomach contents samples (e.g. pepsin) may inactivate the biosensors. Increasing the pH of the stomach contents sample to seven alleviated the toxic effect and would also inactivate any peptic enzymes present.

The *E. coli* biosensor has a high sensitivity towards the PTC's in stomach contents, with EC_{50} values below the concentration that might be expected in stomach contents in rapidly fatal poisonings. A clinically isolated *S. typhimurium* was successfully genetically modified with the *lux* gene set and proved a useful tool for screening stomach contents for the presence of toxic compounds. Initially it was thought that STM biosensor may be more ecologically appropriate for stomach contents testing as *Salmonella* sp. are able to survive passage through the stomach. Although there were differences in the response of the two biosensors to the PTC's tested, both were sensitive enough to detect most of the PTC's (with the exception of cyanide which was not detectable using the STM biosensor). This does demonstrate however, that different species may respond differently to different PTC's and that toxicity screening should, therefore, include more than one test organism. Indeed, it might also be beneficial to include both prokaryotic and eukaryotic organisms to obtain a fuller picture of toxic compounds present and reduce the risk of false negative results. This approach has been used successfully in environmental studies [14–16]. Metabolic and catabolic genetically modified bacterial biosensors were used by Bhattacharyya et al. [14] to assess contaminated ground-water beneath a former airfield. One of the metabolic biosensors used was the same as in this study (*E. coli* HB101), the luminescence of the catabolic biosensors used were only induced in the presence of a specific contaminant, achieved by linking the *lux* genes to a degradation pathway [18]. The biosensors used showed different sensitivities to each other and to the groundwater samples tested. Dalzell et al. [17] used a combination of five toxicity tests including one luminescent micro-organism (*V. fischeri*), they found that the tests used in combination were more effective than using only one test. Horswell et al. [16] used fungal and bacterial biosensors to assess toxicity in soils contaminated with high levels of copper, zinc and nickel. They found that the fungal biosensor detected the high copper and the bacterial biosensor detected high zinc. Thus, if only one biosensor had been used potential toxicity at the site may have been missed.

The toxicity of the PTC's tested in this study was greatly reduced in stomach contents compared to water (and urine, [1]), this is probably because the PTC's may interact directly or indirectly with components of the stomach contents reducing the effective concentration of the PTC available to the biosensor. Stomach contents may contain partially digested food and gastric secretions that include enzymes (pepsin), hydrochloric acid, mucus and intrinisic factor [10]. Indirect effects on PTC's in stomach contents such as ionization can be caused by fluxes in pH [19,9]. Direct interactions with stomach contents such as binding of PTC to partially digested food may also decrease the effective concentration of the PTC and lower the sensitivity of the biosensors. Similar interactions and consequent reductions in toxicity have been demonstrated in environmental testing [15,11,20]. Bundy et al. [15] used microbial biosensors to assess the ecological toxicity of organotins. They found that toxicity to a *lux*-marked *Pseudomonas fluorescens* biosensor was decreased by an order of magnitude in soil extracts as compared to distilled water, due to binding of the organotins to the soluble colloids in the soil solutions [15]. Despite reductions in sensitivity of the biosensors in this study to PTCs in stomach contents, both biosensors were still able to indicate the presence of PTCs tested because the concentration of poisons is generally very high in stomach contents (when compared to urine) (Table 2). Methanol extraction was tested as a possible method of overcoming decreases in sensitivity of the biosensors to PTC's in stomach contents. For most of the PTC's tested, the EC_{50}'s obtained with methanol extraction were not markedly different from direct exposure to the PTC's in stomach contents. However, methanol extraction could be an effective means of determining if a toxic compound is organic.

Initially sensitivity of the biosensors to OTC drugs was carried out in water and the majority of the OTC's tested were toxic to the biosensors at concentrations encountered in stomach contents at therapeutic doses. However, when the stomach contents were spiked with the same concentrations of OTC drugs, no toxicity was detected at levels likely to be encountered in this medium. As with the PTC's, the OTC drugs were probably binding to some component of the stomach contents, reducing the concentration available to interact with the biosensors.

Caffeine is found in beverages such as coffee, tea, cocoa and cola drinks as well as many foods, OTC and prescription drugs. Results from this study suggest that compounds in tea and coffee other than caffeine contribute significantly to toxicity to the biosensors as the EC_{50} values of caffeine in tea and coffee are markedly lower than the EC_{50} value for pure caffeine (Table 3). In stomach contents, the biosensors are less sensitive to tea, indicating that if a subject drank a cup of tea before they died, this would not interfere with the bioassay. Results in stomach contents with coffee however, indicate that the toxicity remains significant despite the probable binding of caffeine (and its subsequent decrease in bioavailability) as indicated by experiments with caffeine alone. This suggests that there are

compounds in coffee apart from caffeine, which are having an effect on the metabolic activity of the biosensor (i.e. exerting a toxic effect). Coffee contains a complex mixture of chemical components of the bean, some of which are not affected by roasting. Other compounds, particularly those related to the aroma, are produced by partial destruction of the green bean during roasting. Non-volatile components include caffeine, trigonelline, chlorogenic acid, phenolic acids, amino acids, carbohydrates, and minerals, and volatile components, which contribute to the aroma of coffee, include organic acids, aldehydes, ketones, esters, amines, and mercaptans [21]. The toxicity of components present in coffee (other than caffeine) have not been tested in this study, but results suggest that their availability to the biosensors does not appear to have been affected by their addition to stomach contents. The caffeine content of tea and coffee depends on the type of product used and also in the way it is prepared (e.g. instant versus filter for coffee). Caffeine would be easily identified by standard toxicological tests and therefore easily be eliminated as the cause of toxicity in the test results. Other compounds found in coffee that appear to exert a toxic effect on the biosensors require further investigation.

5. Conclusions

In this preliminary study, the *E. coli* and STM bacterial biosensors were able to indicate the presence of several commonly encountered organic and inorganic poisons in stomach contents in a 1.25 h screening bioassay (1 h sample preparation followed by 15 min biosensor exposure). A wider variety of PTCs would need to be studied in order to fully validate the potential usefulness of bacterial biosensors as a screening test for a broad range of PTC's in toxicological samples. If fully validated, the biosensor assays could be used to reduce the number of specific and time-consuming tests required to screen for individual PTC's. The variability in response between the two different biosensors suggests that toxicological screening should include more than one test organism to avoid false negative results. The use of a suite of biosensors would not markedly affect the rapidity of the biosensor screening as all the biosensors used in this current study respond to PTC's within 15 min. The majority of OTC compounds if present in the sample, will not interfere with the biosensor response. However, there is a possibility of false positives with Aspirin and coffee.

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